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TETRAPLOIDY IN A HYBRID LIZARD OF THE GENUS *CNEMIDOPHORUS* (TEIIDAE)

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Abstract. An apparent hybrid between triploid, parthenogenetic *Cnemidophorus exsanguis* and diploid, sexual *C. inornatus* is shown to possess a tetraploid chromosome complement. Evidence suggests that this tetraploid karyotype resulted from the fusion of a haploid sperm pronucleus of *C. inornatus* with an egg pronucleus carrying the unreduced triploid chromosome complement of *C. exsanguis*. Observations of captive *C. inornatus* males show their propensity to engage in mating behavior with *C. exsanguis* parthenogenones. These findings are discussed with regard to the origin and genetic mechanism of parthenogenesis in *Cnemidophorus*.

INTRODUCTION

The hypothesis that parthenogenetic species of *Cnemidophorus* arose from the offspring of interspecific hybridizations (Lowe and Wright, 1966) has been supported by evidence from studies of lactate dehydrogenase (Neaves and Gerald, 1968) and adenosine deaminase (Neaves, 1969). A particular aspect of that hypothesis, namely, that triploid parthenogenones originated in crosses between males of a sexual species and females of a diploid, parthenogenetic species, has received support from reports that such hybridizations occur in nature. Taylor and Medica (1966) described apparent hybrids between *Cnemidophorus inornatus*, a sexual species, and *C. neomexicanus*, a diploid parthenogenone.

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Wright and Lowe (1967a) verified the occurrence of *C. inornatus* x *C. neomexicanus* hybrids, conveniently designated *C. "perplexus,"* at several localities in New Mexico where the two parental species are sympatric.

The question arises as to whether or not hybridization in *Cnemidophorus* ends with the attainment of triploidy. Although triploid parthenogenones are common (of seven parthenogenetic species in the *sexlineatus* group, five are triploid), no tetraploid species are known in the genus. Lowe and Wright (1966) mention a suspected tetraploid individual that they considered to represent a hybrid of *C. inornatus* and *C. uniparens,* the latter a triploid parthenogenone, but no evidence, karyotypic or otherwise, was presented to verify their suspicion. My report describes in detail a documented instance of tetraploidy in a *Cnemidophorus* hybrid and seeks to explain why there are as yet no tetraploid species in nature. Observations of interspecific mating behavior involving male *C. inornatus* and females of various parthenogenetic species are included in this report. Finally, these findings are discussed with regard to the origin and genetic mechanism of parthenogenesis in *Cnemidophorus.*

MATERIALS AND METHODS

Animals used in these studies were captured alive in their native habitats in Colorado, New Mexico, and Texas during the summers of 1967 and 1968. Soon after capture, these lizards were shipped to Boston and Cambridge. Upon arrival in the laboratories, they were housed either in a rectangular pen 12 feet by 14 feet or in oval pens 3.5 feet by 5 feet. The pens had sand floors with tiles and sheets of cardboard scattered about for shelter. Sun lamps installed over the pens were automatically controlled to provide a twelve-hour daily photoperiod. A constant supply of open water and available food, in the form of mealworms and crickets, was provided.

Aspects of lizard behavior reported here were observed and photographed among the captive inhabitants of the large rectangular pen described above. Most incidents were recorded during the winter of 1968–1969 when the pen held approximately 150 *Cnemidophorus* distributed among five sexual and five parthenogenetic species.

The tetraploid karyotype illustrated in this report was obtained from a phenotypically aberrant lizard captured at Alamogordo, New Mexico, in August, 1967. This lizard was maintained alive until May, 1968, when it was used for karyotypic study. Mitotic figures were obtained from tissue cultures of 1 mm cubes of heart tissue according to the following procedure. Small Falcon flasks were loaded with 5.0 ml of a mixture containing 80 percent Eagle's Minimum Essential Medium (Microbiological Associates) and 20 percent fetal calf serum. With the flask in an upright position, tissue explants were placed against the dry wall of the flask. The flask was slowly lowered to its side so that the explants were covered by the medium but not dislodged from the plastic surface. The flasks were incubated at 33° C. without agitation. The cultures were examined regularly with an inverted phase contrast microscope in order to follow cell proliferation. On the ninth day of incubation, when mitotic activity was intense, 0.25 microgram of Velban (Grand Island Biological Co.) was added to each flask. Twenty-four hours later, the medium was discarded and 5.0 ml of 0.1 percent trypsin in 0.8 percent sodium citrate was added to each flask. Following a twenty-minute incubation at 37° C., the flasks were shaken vigorously to free the dissociating cells from one another and from the plastic. From this point, the cultures were processed according to a modification of the method outlined by Moorhead *et al.* (1960). The solution from each flask was decanted into a centrifuge tube and spun at 1000 g for five minutes. The cell pellet was fixed in two changes of ethanol:acetic acid (3:1) and was suspended in 45 percent acetic acid. Drops of this suspension were placed on cold, wet slides and flame dried. The chromosomes on these slides were stained with 1 percent toluidine blue in 1 percent borax and were photographed with an Olympus photomicroscope.

THE DISCOVERY OF THE TETRAPLOID

In the course of a collecting trip to New Mexico in August, 1967, I visited an exceptionally dense *Cnemidophorus* population within the city limits of Alamogordo in Otero County. The population was largely restricted to a weed bed approximately 20 meters wide and over 1000 meters long, bounded on the west by the roadbed of the Southern Pacific Railroad and on the east

by the Alamogordo City Park. Two *Cnemidophorus* species were found in the area described. *C. exsanguis,* a triploid parthenogenone, was most abundant, occurring at a density of approximately 50 animals per acre; *C. inornatus,* a sexual species, occurred at a density of about 10 animals per acre. No lizards were seen in the park itself, which had a cover of closely mown grass. West of the roadbed, the cover consisted of mesquite shrub and cactus on an eroded surface virtually devoid of weeds or grass. Here both *C. inornatus* and *C. tigris,* a second sexual species, were abundant. In two days of collecting west of the roadbed, only a single *C. exsanguis* was seen. No *C. tigris* were found east of the roadbed in the weeds where *C. exsanguis* was so abundant.

Forty-three *C. exsanguis* and eleven *C. inornatus* were captured in the Alamogordo weed bed in 1967. In addition, two aberrant *Cnemidophorus* were taken. One of these, MCZ*100080, was the size of *C. inornatus,* with partially fused paravertebral stripes, a white ventral surface, and a rusty tint on its dorsum, causing it to resemble *C. exsanguis.* The day after its capture, this animal died. Its abdomen was opened to expose the gonads, which appeared rudimentary. The nature of this specimen remains a mystery. A second aberrant specimen, MCZ 101991 (Plate 1), resembled a typical *C. exsanguis* except that its paravertebral stripes were fused and its ventral surface and tail were suffused with a brilliant blue characteristic of the same surfaces in *C. inornatus* males. This *exsanguis*-like animal was maintained alive for almost nine months before it was sacrificed for a study of its chromosomes.

It was immediately suspected that MCZ 101991 might represent a cross between the sympatric species of the weed bed, *C. inornatus* and *C. exsanguis.* This lizard clearly possessed attributes characteristic of both suspected parental species. A decision was reached to allow the animal to remain alive as long as possible so that its behavior might be observed, but at the same time, an assessment of its ploidy was desired. Accordingly, blood smears were prepared from the *exsanguis*-like animal and from other *Cnemidophorus* known to be either diploid or triploid on the

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basis of karyotypic evidence (Lowe and Wright, 1966; Wright and Lowe, 1967b). These were sent to N. B. Atkin for densitometric estimation of DNA in the nucleated erythrocytes. Dr. Atkin reported (in litt.) that karyotypically triploid species ($3n = 69 \pm 1$) possessed about 50 percent more DNA per nucleus than karyotypically diploid species ($2n = 46$) and that the aberrant specimen (MCZ 101991) possessed about 20 percent more DNA per nucleus than triploids such as *C. exsanguis*. The details of erythrocyte DNA analysis in *Cnemidophorus* will be reported elsewhere. Although a true tetraploid should have demonstrated approximately 30 percent more DNA per nucleus than a triploid, the results of Atkin's survey encouraged the suspicion that the aberrant specimen represented a hybrid carrying some genetic elements characteristic of *C. inornatus* in addition to the basic triploid complement characteristic of *C. exsanguis*.

In December 1967, Professor C. H. Lowe examined the aberrant lizard and declared it unlike any *Cnemidophorus* he had ever seen. Lowe agreed that it might represent a tetraploid hybrid between *C. inornatus* and *C. exsanguis*. Meanwhile, this lizard thrived in captivity and proved itself a vigorous competitor for food; it commonly robbed typical *C. exsanguis* of mealworms.

In January 1968, the aberrant lizard laid two eggs, thus confirming the suspicion that it was female and suggesting that its ovaries were functional. Attempts were made to provide suitable conditions for ovoposition, but she did not take advantage of them, and the eggs were dessicated by the time I discovered them in my daily check. She produced no more eggs during her captivity, which extended through May, 1968.

When the aberrant lizard was eventually sacrificed for karyotypic analysis, the results (Plates 2 and 3 and Table 1) confirmed its suspected status as a tetraploid hybrid. The modal chromosome number, based on examination of eight apparently complete chromosome spreads, is 91. The karyotype in Plate 2 is from the single spread showing 92 chromosomes. Considering the large number of chromosomes in the karyotype, the small size of many, and the probability of overlap, it is not surprising that fewer than 92 chromosomes are evident in most spreads. The tetraploid karyotype can be divided into four apparently identical haploid complements, each closely resembling the haploid chromosome

complement of *C. inornatus* (Lowe and Wright, 1966). Three *inornatus*-like haploid sets constitute the typical triploid karyotype of *C. exsanguis,* which is similar to that of another triploid parthenogenone, *C. uniparens* (Lowe and Wright, 1966). The fourth haploid chromosome complement seen in the aberrant lizard's karyotype may have been contributed by *C. inornatus* itself, through an interspecific mating with *C. exsanguis.*

OBSERVATIONS OF CAPTIVE SPECIMENS: INTERSPECIFIC MATING

Observations of captive *Cnemidophorus* during autumn and winter of 1968–1969 suggest that *C. inornatus* males may be unusually disposed to interspecific mating with parthenogenetic females. Dozens of courtship encounters, including attempted copulation, were recorded between *C. inornatus* males and parthenogenetic females from the species *C. exsanguis* and *C. tesselatus* (Plate 4, figs. 5–9).

The sequence of sexual behavior involved pursuit of a subject, often a parthenogenetic female but sometimes an individual of a sexual species, including other *C. inornatus,* for five to twenty seconds at a speed depending on the movement of the subject, the male apparently trying to stay near the tip of the subject's tail. Suddenly, the *C. inornatus* male would close with the subject, mount its back, and grasp in his jaws a fold of skin on the back of the subject's neck. If the subject acquiesced, the male would immediately execute sinuous movements while pressing its abdomen against the subject's dorsum. With the subject still quiescent, the male would loosen its grip on the subject's skin and caress the back of the subject's head and neck with his jaw while continuing the sinuous body movements. The male seemed particularly intent on rubbing his ventral pelvis and tail base against the dorsal pelvis of the subject. Meeting no resistance, the male would maneuver his vent into the area between the subject's rear limb and tail base. When the subject was a large parthenogenetic female, this maneuver required the male to creep back from his original position in which his jaw touched the subject's head and neck (Plate 4, figs. 5–7). While struggling to approximate his vent to the subject's, the male would constantly stroke the subject's dorsal pelvis and tail base with his rear limb. At times, the

male's hemipenis was visibly erect, but in spite of observed close apposition of the participants' vents, unequivocal intromission was not seen.

It did not appear as though *C. inornatus* males met with any real cooperation, beyond mere quiescence, in their courtship efforts, but *C. exsanguis* and *C. tesselatus* at least permitted the sequence of events to proceed as far as described above. Other *C. inornatus,* particularly other males, made real efforts to escape the attentions of an ardent but misguided suitor. Perhaps as a result, *C. inornatus* males exhibited a definite preference for the larger, parthenogenetic females over their conspecific females. Attempted intromission was observed only between *C. inornatus* males and parthenogenetic females.

DISCUSSION

The existence of a tetraploid lizard in a mixed population containing triploid parthenogenones and diploid, sexual lizards argues that tetraploid hybrids are synthesized in nature, although the genus *Cnemidophorus* apparently contains no tetraploid species.[1] That the tetraploid lizard described here is a hybrid and not an autopolyploid is suggested by its possession of phenotypic traits characteristic of both suspected parental species, by the propensity of the suspected male parent, *C. inornatus,* to engage in interspecific mating behavior with parthenogenetic females such as *C. exsanguis,* and by the occurrence in nature of another interspecific hybrid, *C. "perplexus,"* which is considered to be the result of crosses between sexual and parthenogenetic lizards (Wright and Lowe, 1967a).

Two questions are raised by the existence of the tetraploid hybrid. First is the question of the cytological events in gametogenesis and fertilization that permit a tetraploid to arise from a mating between a sexual male and a triploid, parthenogenetic female. This question, which is essentially concerned with genetic mechanisms operative in parthenogenesis, will be considered at some length in order to provide a background for the second question. The second question concerns the absence of tetraploid species in spite of the synthesis of hybrid tetraploids in nature.

The first question has a trivial aspect, namely cytological events in the sexual male. Nothing more is required of the male lizard

[1] See also the discussion of two tetraploid lizards in a mixed population of *C. sonorae* and *C. tigris* (Lowe et al., 1970).

than the production of typical sperm that carry a haploid chromosome complement and that are capable of delivering this genome to the female pronucleus in a mature egg. A more complicated aspect of the question involves the cytological events that lead to the production of a mature egg whose pronucleus contains the unreduced somatic chromosome complement characteristic of the mother. This, in essence, is the question of the mechanism of parthenogenesis in *Cnemidophorus,* and it deserves detailed consideration.

In *Cnemidophorus,* with the possible exception of *C. lemniscatus* (Vanzolini, 1970; Hall, 1970), interspecific hybridization is implicated in the origin of parthenogenesis. Oogenesis in parthenogenones arising from interspecific hybridization must solve two major problems: 1) the maneuvering of potentially, and sometimes obviously, nonhomologous chromosome sets at meiosis, and 2) the production of a mature egg possessing the somatic chromosome number characteristic of the mother. Several solutions have been proposed: 1) mitotic egg production replaces the normal meiotic mechanism (Lowe and Wright, 1966), 2) failure of either first or second polar body formation, or fusion of one of these bodies with the egg nucleus after its formation (Beatty, 1957), 3) suppression of the first cleavage division (Beatty, 1957), or 4) endomitosis in the oogonium prior to the onset of meiosis (MacGregor and Uzzell, 1964).

Solutions 2 and 3 cannot compensate for difficulties in bivalent formation that arise when nonhomologous chromosome sets are present at the onset of meiosis, nor can they compensate for situations in which more than two homologous chromosome sets enter meiosis. Since some diploid parthenogenones, such as the four *Lacerta* parthenogenones studied by Darevsky (1966), may be derived from closely related sexual species possessing fairly homologous karyotypes, solutions 2 and 3 cannot be ignored. In fact, Darevsky's (1966) study of parthenogenetic *Lacerta* suggests that the second meiotic division is incomplete, with a diploid restitution nucleus resulting from the fusion of the daughter pronuclei during anaphase. Solutions 2 and 3 can, however, be discounted in all self-perpetuating triploids, such as *C. exsanguis,* and in diploids containing grossly nonhomologous haploid chromosome complements, such as *C. neomexicanus.* If only solution 2 or 3 were operative in these cases, bivalent formation should fail,

owing to the absence of homologues, or, in those triploids that appear to have homologous haploid chromosome sets, owing to a tendency toward trivalent formation.

Solution 1, mitotic egg production, overcomes difficulties in bivalent formation by avoiding the issue completely. This solution suffers from a lack of supporting evidence; no instance is known in which the germ line of a higher organism can facultatively abandon the meiotic theme. Furthermore, solution 1 is rendered dubious in *Cnemidophorus,* the group for which it was originally proposed (Lowe and Wright, 1966), by evidence (personal observations) that the nuclei of oocytes approximately 1 mm in diameter from ovaries of parthenogenetic species such as *C. exsanguis, C. neomexicanus,* and *C. tesselatus* contain bivalent lampbrush chromosomes characteristic of meiotic diplotene. Hence, at least some components of typical meiosis occur in the ovaries of parthenogenetic *Cnemidophorus.*

Solution 4, pre-meiotic endomitosis in the oogonia, has been shown to work in one group of parthenogenetic vertebrates, the ambystomatid salamanders (MacGregor and Uzzell, 1964), and appears likely in triploid poecilid fish (Schultz, 1967). Since pre-meiotic endomitosis can solve the problems of increased ploidy and nonhomologous chromosome complements, it is the most probable mechanism operative in parthenogenetic *Cnemidophorus* as well.[1] This probability justifies discussion of its known features and its genetic implications.

While studying preparations of diplotene lampbrush chromosomes, MacGregor and Uzzell (1964) found 42 bivalents in each germinal vehicle from triploid parthenogenones ($3n = 42$), but in germinal vesicles from closely related, diploid sexual species ($2n = 28$), they found the expected 14 bivalents. This finding was explained by the postulation that endomitosis precedes meiosis in parthenogenetic oocytes, so that such oocytes enter meiosis with a hexaploid ($6n = 84$) chromosomal constitution. Meiotic DNA replication would then give an oocyte that is dodecaploid with respect to DNA, and meiosis would operate on

[1] A recent paper by Orlando Cuellar verifies the occurrence of this mechanism in the triploid parthenogenone *Cnemidophorus uniparens.*

42 tetrads (bivalents) to yield a pronucleus with 42 chromosomes, which is the somatic complement for triploid ambystomatids.

MacGregor and Uzzell (1964) suggest that only sister chromosomes resulting from the endomitotic duplication associate to form bivalents. The genetic status of all parthenogenones that may rely on pre-meiotic endomitosis hinges on the question of bivalent formation, making a critical assessment of the MacGregor-Uzzell proposition necessary. The method of bivalent formation will determine whether the parthenogenetic lineage will retain the heterozygosity inherent in its hybrid origin or will experience an ultimate tendency toward homozygosity. While this question is not directly relevant to the issue of tetraploid hybrids, it is important to an understanding of parthenogenesis in *Cnemidophorus*.

Should sister chromosomes pair exclusively, a most conservative pattern of inheritance would result; each offspring would, neglecting mutation, emerge with an exact copy of its mother's genome. Should homologous chromosomes also pair, independent assortment at first meiotic metaphase would cause the offspring to deviate from the mother's genotype, owing to the loss of alternative alleles in the first polar body. In triploid parthenogenones relying on pre-meiotic endomitosis, the probability of losing both representatives (sisters) of a single chromosome from a homologous set of three in a single generation can be calculated ($p = 0.066$), assuming random pairing of sister and homologous chromosomes, no crossing-over, and independent assortment. Similarly, after only two generations, $p = 0.0066$ that two chromosomes from a homologous set of three will be lost, leaving complete homozygosity at all loci on that chromosome.

A diploid parthenogenone in which sister and homologous chromosomes pair randomly experiences an even stronger tendency toward homozygosity. The probability is 0.33 that one of a set of two homologous chromosomes will be lost in a single generation if crossing-over does not occur. In either diploidy or triploidy, crossing-over will only randomize the occurrence of homozygosity with respect to all loci on a single chromosome and will not delay the trend of the entire genome toward homozygosity. The ultimate consequence of participation of homologous chromosomes in bivalent formation is homozygosity at all loci in the genome.

The most sensitive test devised to assess the genetic status of

parthenogenones, namely tissue grafts to determine histocompatibility (Kallman, 1962; Maslin, 1967), does not discriminate between uniform clonal heterozygosity, which would be preserved in the case of exclusive pairing of sisters, and established clonal homozygosity resulting from independent assortment of bivalents formed from random pairing of both sister and homologous chromosomes. Only the transient period of developing homozygosity characteristic of the latter situation would be revealed as frequent failure of parent-to-offspring grafts and sibling-to-sibling grafts.

In order to judge if pairing is restricted to sisters, as suggested by MacGregor and Uzzell (1964), one is left with the task of examining directly the composition of bivalents, or of inferring the degree of homo- or heterozygosity that a given parthenogenetic clone might possess. The former possibility, direct determination of bivalent composition, is simple enough in principle. The administration of ^{3}H-thymidine to a parthenogenone at the synthetic phase of mitosis immediately preceding endomitosis would result in the presence of radioactive label in one member of each sister pair arising from endomitotic duplication. Autoradiography of lampbrush bivalents would then show label in half of each bivalent, should strict sister pairing be the rule. Random pairing of sisters and homologues would result in some bivalents unlabeled, some half labeled, and some wholly labeled. Crossing-over would not complicate interpretation. Although such an experiment would clearly resolve the question of bivalent composition, practical problems, such as finding the proper time in the animal's life cycle for ^{3}H-thymidine administration, make this a difficult exercise.

Judgement of the degree of homo- or heterozygosity in a parthenogenetic clone, and hence, inference of the composition of meiotic bivalents, can be based on studies of phenotypic variation. While some good studies of phenotypic variation in parthenogenones and in their sexual relatives have been performed (Zweifel, 1965), results are not easily interpreted in favor of either homo- or heterozygosity. For example, groups of *C. tesselatus* from a given locality were found to exhibit a range of variation in many characters that approximated half that seen in local populations of the sexual lizard, *C. tigris* (Zweifel, 1965). Does the relatively

smaller variation seen in parthenogenones reflect the existence of homozygous clones, or does it indicate the importance of recombination in freeing variation inherent in heterozygous genomes? Complicating this question is a fundamental ignorance of the genetic regulation of most phenotypic expression. One could resort to the doctrine of superior fitness in heterozygotes and argue that parthenogenones that compete successfully with their sexual counterparts must necessarily be heterozygous. This begs an interesting question and ignores an opportunity to test notions of fitness that have become a foundation of evolutionary theory. Furthermore, such reasoning is contradicted by Darevsky's (1966) observations on the cytology of parthenogenesis in *Lacerta*. Darevsky maintains that failure of second meiotic division is the parthenogenetic mechanism operative in these lizards. Under this mechanism, crossing-over will temporarily maintain some heterozygosity, particularly at loci far from the centromere. However, the ultimate tendency is toward complete homozygosity of the genome (Beatty, 1957). Hence, if Darevsky's observations are correct, one must expect the competitively successful *Lacerta* parthenogenones to exhibit a high degree of homozygosity relative to their sexual counterparts.

Assuming that pre-meiotic endomitosis facilitates parthenogenesis in *Cnemidophorus*, a reliable indication that pairing may be strictly limted to sister chromosomes comes from studies that have deduced genotypes for certain enzymes. Parthenogenetic *Cnemidophorus* exhibit a striking incidence of heterozygosity at genetic loci determining phenotypes for lactate dehydrogenase, adenosine deaminase, phosphogluconate dehydrogenase, and NADP-dependent malate dehydrogenase (see Table 1 in Neaves, 1969). Most impressive are the genotypes for adenosine deaminase; every parthenogenetic species studied showed heterozygosity at the *ada* locus. This is clear evidence in favor of fixed heterozygosity in parthenogenetic *Cnemidophorus*, and hence, in favor of strict sister pairing at meiosis.

With the question of parthenogenetic mechanisms aired, it appears that pre-meiotic endomitosis provides a basis for understanding how a triploid, parthenogenetic *C. exsanguis* could produce an egg whose pronucleus carried an unreduced somatic chromosome complement, and how the union of this pronucleus

with a haploid pronucleus from male *C. inornatus* could result in an offspring carrying the chromosomes seen in Plates 2 and 3. A precedent for these events is established in the occurrence of such a fertile union of pronuclei from parthenogenetic females and sexual males in the genus *Poecilia* (Rasch *et al.*, 1965).

There remains the question of the absence of tetraploid species of *Cnemidophorus* in spite of the existence of tetraploid hybrids in nature. Probing this question requires some indulgence in speculation, which the novelty of the subject will hopefully excuse.

The most likely reason for the absence of tetraploid species may be the failure of tetraploid hybrids to reproduce parthenogenetically. There is no evidence that the tetraploid lizard described in this study was parthenogenetically competent. The fact that it laid fully yolked eggs does not imply that these eggs either, 1) carried an unreduced chromosome complement, or 2) were capable of undergoing spontaneous embryonic development. These are two basic criteria that must be met if an interspecific hybrid is to achieve the reproductive success characteristic of existing parthenogenetic species in the genus *Cnemidophorus*. If one prefers to assume that tetraploid hybrids can reproduce parthenogenetically, then one must account for their absence as species on the grounds that no suitable ecological niche is available to them or that they cannot successfully compete with other species for a mutually suitable niche. However, the similarity of the known tetraploid hybrid to other *Cnemidophorus* suggests that it might compete with them for a currently available niche, and the behavior of the tetraploid lizard in captivity suggests that it could be successful in this regard. It seems that one must attempt instead to justify reproductive failure.

One possibility is that tetraploidy is incompatible with the mechanism of parthenogenesis operative in *Cnemidophorus*. However, the suspected mechanism, which is pre-meiotic endomitosis, has the important virtue of theoretically permitting any karyotype, regardless of ploidy, to function normally at meiosis. Given the suspected mechanism of parthenogenesis, tetraploidy itself should not be a barrier to reproduction.

The most attractive possibility is that only a small proportion of interspecific hybrids meets the basic requirements of parthenogenetic reproduction. In other words, the genetically determined

compensatory events, such as pre-meiotic endomitosis and spontaneous embryonic development, which are presumably needed for parthenogenesis, may be frequently absent in F_1 hybrids. This possibility is particularly attractive, owing to the apparent genetic uniformity within existing parthenogenetic species of *Cnemidophorus*.

Maslin (1967) has demonstrated a pattern of histocompatibility in *C. tesselatus* that suggests that all diploid members of the species, even when taken from localities hundreds of miles apart, are genetically identical. Similarly, all triploid *C. tesselatus* are reciprocally histocompatible, and what is more, can accept tissue grafts from the diploids but cannot reciprocate. Biochemical evidence (Neaves, 1969) suggests that diploid *C. tesselatus* arose from interspecific hybridization between *C. tigris* and *C. septemvittatus,* two sexual species, and that triploid *C. tesselatus* resulted from the addition of a haploid genome from *C. sexlineatus,* a third sexual species, to the diploid *C. tesselatus* genome. Coupled with this evidence, Maslin's (1967) findings suggest that all existing populations of *C. tesselatus* arose from the offspring of a single hybrid individual representing a cross between *C. septemvittatus* and *C. tigris* and that all triploid *C. tesselatus* are derived from a single hybrid lizard representing a cross between *C. sexlineatus* and diploid *C. tesselatus*. The genetic uniformity in *C. tesselatus* could not exist if the species contained offspring of more than one parthenogenetic hybrid, since each individual hybrid resulting from a *C. septemvittatus* x *C. tigris* cross will carry a unique recombinant genotype.

The genetic uniformity of *C. tesselatus* points to either one or both of two possibilities, namely that interspecific hybrids are rare or that parthenogenetic competence in an interspecific hybrid is rare. The first possibility cannot be ruled out in the case of *C. tesselatus,* as no evidence is available that might suggest the frequency at which hybrids between either *C. septemvittatus* and *C. tigris* or *C. sexlineatus* and diploid *C. tesselatus* occur in nature. The first possibility can be eliminated in the case of *C. "perplexus,"* the hybrid between *C. inornatus* and *C. neomexicanus*. *C. "perplexus"* was first collected in New Mexico in 1841 (Maslin *et al.,* 1958), and since 1962, at least six of these hybrids have been captured at sites where both *C. inornatus* and *C. neomexicanus*

are sympatric (Wright and Lowe, 1967a). This is a case where hybrids are rather common in nature and where they have occurred for at least 130 years without developing a parthenogenetic species. This case supports the view that genetic uniformity in existing parthenogenetic species and the absence of tetraploid species are both due to the rarity with which parthenogenetic competence is achieved in interspecific hybrids.

The establishment of a parthenogenetic species in *Cnemidophorus* may require a lengthy period of experimentation in which thousands or more individual hybrids are synthesized before a reproductively successful hybrid gene combination occurs. Nevertheless, the result may still be termed saltatory speciation, in that the divergence of the new species from its progenitors is instantaneous, deriving as it does from a single, reproductively fit individual, rather than from cumulative changes in a population over long periods of time. It seems that both *C. "perplexus"* and the tetraploid hybrid illustrated here could represent previews of species that might eventually become established in the New Mexico deserts, if a gene combination facilitating parthenogenetic reproduction ultimately occurs in one of these hybrids.

SUMMARY

A tetraploid lizard resembling *C. exsanguis* but bearing traits characteristic of *C. inornatus* is considered to have resulted from a hybrid mating in which a haploid sperm pronucleus of *C. inornatus* fused with an egg pronucleus carrying the unreduced somatic chromosome complement of *C. exsanguis,* a triploid parthenogenone. Production of such an egg by *C. exsanguis* may have relied on endomitosis in the oogonium, followed by normal meiosis operating on bivalents composed of paired sister chromosomes. This modification of oogenesis is compatible with all known aspects of parthenogenesis in *Cnemidophorus,* including the existence of apparently fixed heterozygosity within parthenogenetic species.

Most cases of parthenogenesis in *Cnemidophorus* began with the synthesis of interspecific hybrids. However, several considerations suggest that many hybrids may be generated before a parthenogenetically competent individual, capable of giving rise to a species, is produced. Among these suggestive considerations

are: 1) the synthesis of many hybrid individuals in nature, i.e., *C. "perplexus,"* without the appearance of a corresponding species, and 2) the apparent genetic uniformity of parthenogenetic species, which indicates their origin from a single hybrid individual. Thus, the appearance of a new parthenogenetic species in *Cnemidophorus* may be preceded by a period of hybridization during which large numbers of reproductively incompetent prototypes of the new species are generated.

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LITERATURE CITED

Beatty, R. A. 1957. *Parthenogenesis and Polyploidy in Mammalian Development.* London, Cambridge University Press.

Cuellar, O. 1971. Reproduction and the mechanism of meiotic restitution in the parthenogenetic lizard *Cnemidophorous uniparens.* J. Morphology, **133**(2): 139–165.

Darevsky, I. S. 1966. Natural parthenogenesis in a polymorphic group of Caucasian rock lizards related to *Lacerta saxicola* Eversmann. J. Ohio Herp. Soc., **5**: 115–152.

Hall, W. P. 1970. Three probable cases of parthenogenesis in lizards (Agamidae, Chamaeleontidae, Gekkonidae). Experientia, **26**: 1271–1273.

Kallman, K. D. 1962. Gynogenesis in the teleost, *Mollienesia formosa* (Girard), with a discussion of the detection of parthenogenesis in vertebrates by tissue transplantation. J. Genetics, **58**: 7–21.

Lowe, C. H., and J. W. Wright. 1966. Evolution of parthenogenetic species of *Cnemidophorus* (whiptail lizards) in western North America. J. Arizona Acad. Sci., **4**: 81–87.

Cole, C. J., and R. L. Bezy. 1970. Natural hybridization between the teiid lizards *Cnemidophorus sonorae* (parthenogenetic) and *Cnemidophorus tigris* (bisexual). Syst. Zool., **19**: 114–127.

MacGregor, H. C., and T. M. Uzzell. 1964. Gynogenesis in salamanders related to *Ambystoma jeffersonianum.* Science, **143**: 1043.

Errata for Breviora 381 by William B. Neaves, lines 35–37, page 16:

_____, _____, C. J. Cole, and R. L. Bezy. 1970. Natural hybridization between the teiid lizards *Cnemidophorus sonorae* (parthenogenetic) and *Cnemidophorus tigris* (bisexual). Syst. Zool., **19**: 114–127.

Maslin, T. P. 1967. Skin grafting in the bisexual teiid lizard *Cnemidophorus sexlineatus* and in the unisexual *C. tesselatus*. J. Exp. Zool., **166**: 137–150.

———, R. G. Beidleman, and C. H. Lowe. 1958. The status of the lizard *Cnemidophorus perplexus* Baird and Girard (Teiidae). Proc. U.S. Nat. Mus., **108**: 331–345.

Moorhead, P. S., P. C. Nowell, W. J. Mellman, B. M. Battips, and D. A. Hungerford. 1960. Chromosome preparations of leucocytes cultured from human peripheral blood. Exptl. Cell Res., **20**: 613–616.

Neaves, W. B. 1969. Adenosine deaminase phenotypes among sexual and parthenogenetic lizards in the genus *Cnemidophorus* (Teiidae). J. Exp. Zool., **171**: 175–184.

———, and P. S. Gerald. 1968. Lactate dehydrogenase isozymes in parthenogenetic teiid lizards (*Cnemidophorus*). Science, **160**: 1004–1005.

Rasch, E. M., R. M. Darnell, K. D. Kallman, and P. Abramoff. 1965. Cytophotometric evidence for triploidy in hybrids of the gynogenetic fish, *Poecilia formosa*. J. Exp. Zool., **160**: 155–170.

Schultz, R. J. 1967. Gynogenesis and triploidy in the viviparous fish *Poeciliopsis*. Science, **157**: 1564–1567.

Taylor, H. L., and P. A. Medica. 1966. Natural hybridization of the bisexual teiid lizard *Cnemidophorus inornatus* and the unisexual *Cnemidophorus perplexus* in southern New Mexico. Univ. Colorado Studies, ser. biol., **22**: 1–9.

Vanzolini, P. E. 1970. Unisexual *Cnemidophorus lemniscatus* in the Amazonas Valley: a preliminary note (Sauria, Teiidae). Papéis Avulsos Zool., S. Paulo, **23**: 63–68.

Wright, J. W., and C. H. Lowe. 1967a. Hybridization in nature between parthenogenetic and bisexual species of the whiptail lizards (genus *Cnemidophorus*). American Mus. Novitates, No. 2286: 1–36.

———, and ———. 1967b. Evolution of the alloploid parthenospecies *Cnemidophorus tesselatus* (Say). Mamm. Chrom. Newslett., 8: 95–96.

Zweifel, R. G. 1965. Variation in and distribution of the unisexual lizard *Cnemidophorus tesselatus*. American Mus. Novitates, No. 2235: 1–49.

Table 1

chromosome number

	87	88	89	90	91	92
frequency	2	0	1	1	3	1

Table 1. Eight apparently complete chromosome spreads from MCZ 101991, the tetraploid hybrid *Cnemidophorus*, were studied. The frequency with which various chromosome numbers were observed in these spreads is indicated in this table.

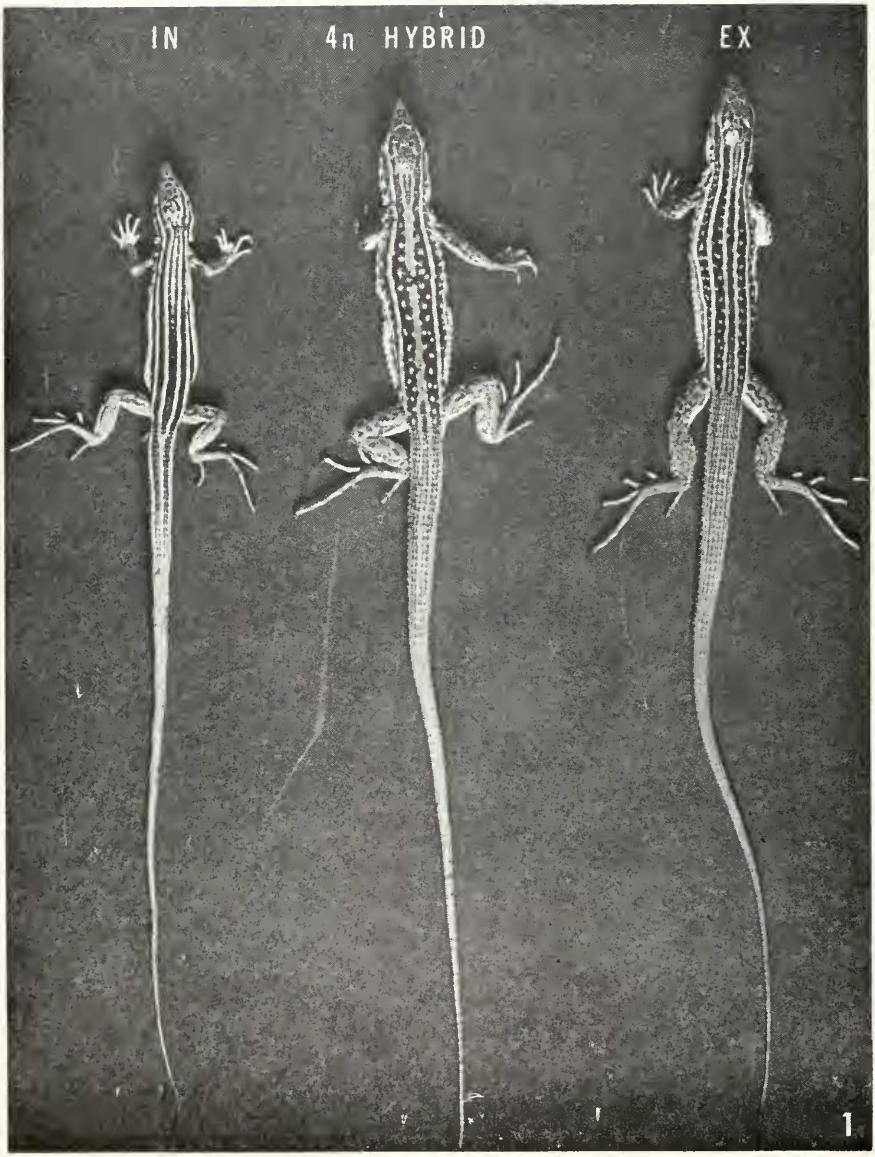
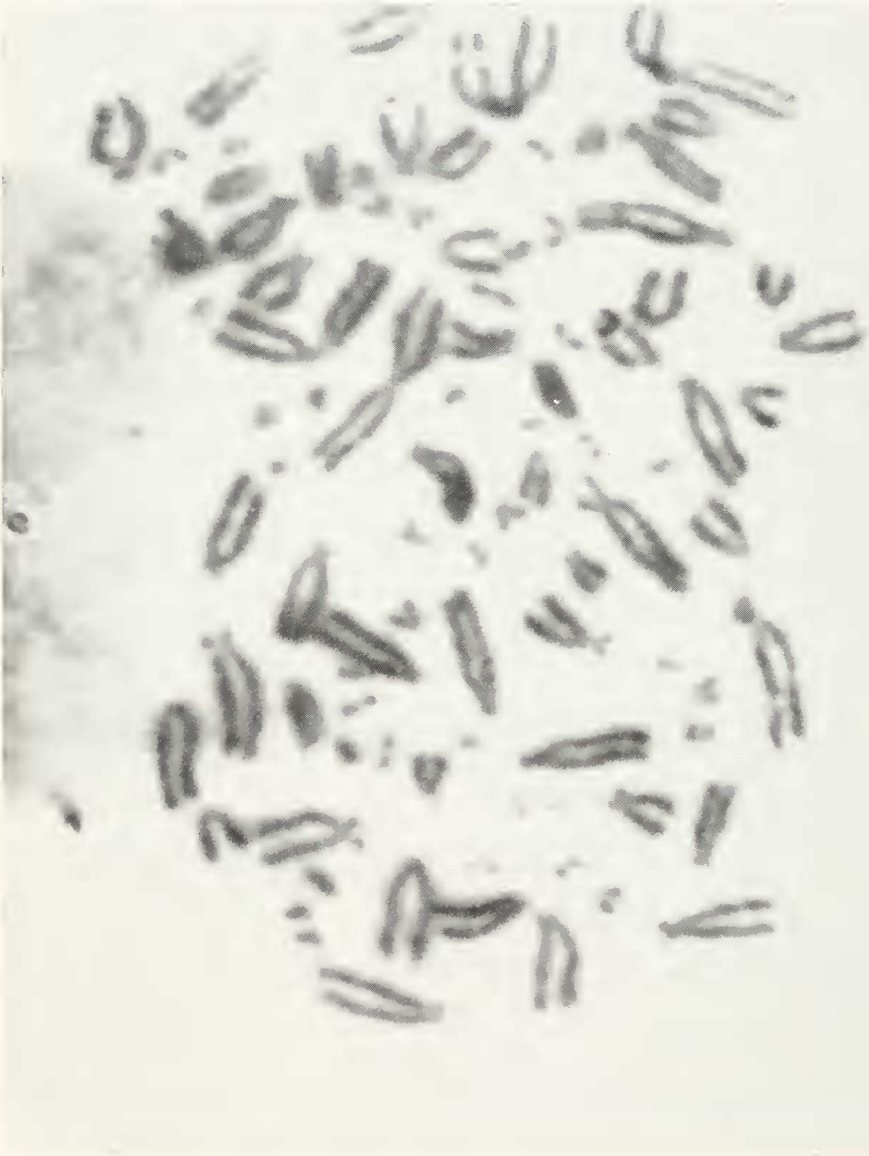

IN
4n HYBRID
EX
1

Plate I

Figure 1. A tetraploid hybrid (MCZ 101991) is shown between its suspected parental species, *C. inornatus* (IN) and *C. exsanguis* (EX). Unlike typical *C. exsanguis*, the hybrid has fused paravertebral stripes and blue on its tail and ventral surfaces. *C. inornatus* also has vivid blue on these surfaces.



2.

Plate II

Figure 2. An apparently complete set of 92 chromosomes from the tetraploid hybrid (MCZ 101991) is shown. The technique for obtaining chromosome spreads is described in the text.

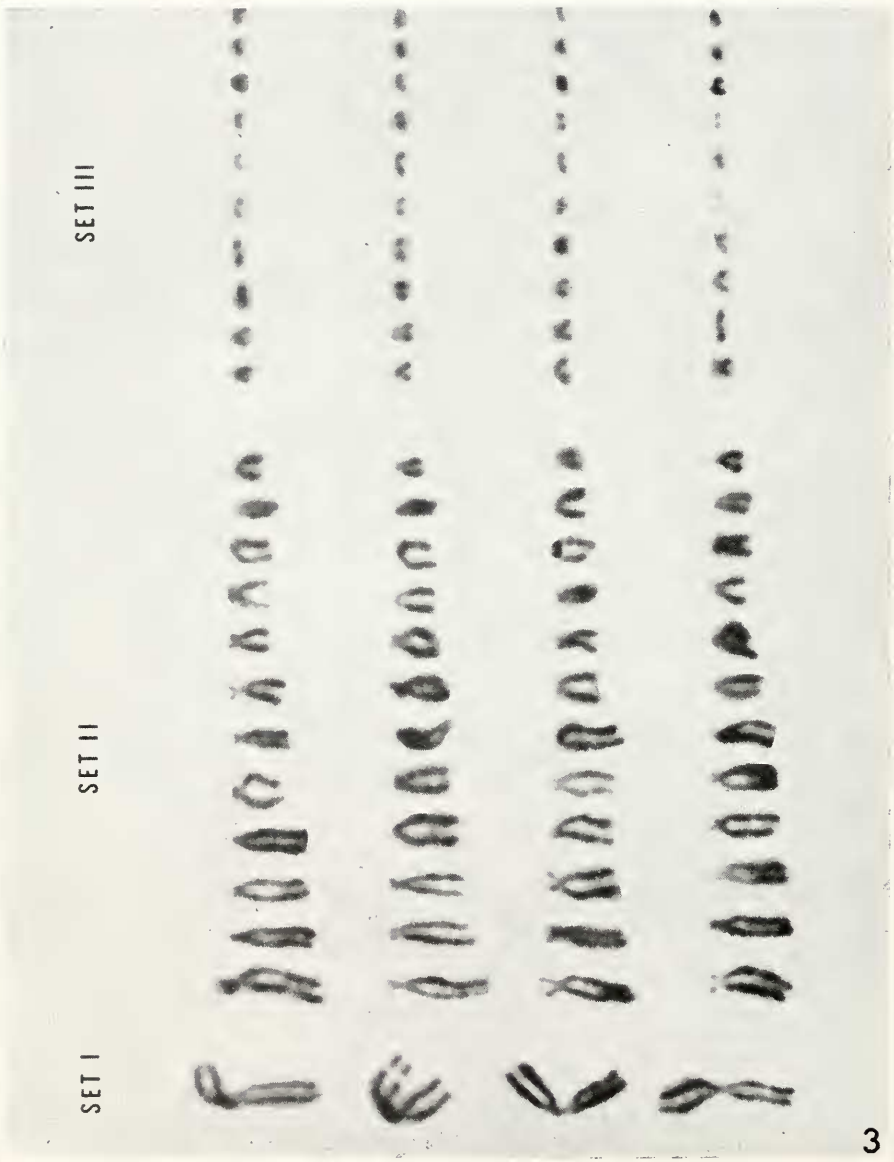

SET I
SET II
SET III
3

Plate III

Figure 3. A karyotype prepared from chromosomes in Fig. 2 is shown. The chromosomes have been sorted into four similar haploid complements, each resembling the haploid chromosome complement of *C. inornatus*. According to established procedure for this genus (Lowe and Wright, 1966), each haploid complement has been subdivided into three sets on the basis of chromosome size and centromere position.